Survey of Susceptibilities to Monosultap, Triazophos, Fipronil, and Abamectin in *Chilo suppressalis* (Lepidoptera: Crambidae)

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ABSTRACT To provide a foundation for national resistance management of the Asiatic rice borer, *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae), a study was carried out to determine dose–response and susceptibility changes over a 5-yr period in the insect from representative rice, *Oryza sativa* L., production regions. In total, 11 populations were collected from 2002 to 2006 in seven rice-growing provinces in China, and they were used to examine their susceptibility levels to monosultap, triazophos, fipronil, and abamectin. Results indicated that most populations had increased tolerance to monosultap. Several field populations, especially those in the southeastern Zhejiang Province, were highly or extremely highly resistant to triazophos (resistance ratio [RR] = 52.57–899.93-fold), and some populations in Anhui, Jiangsu, Shanghai, and the northern rice regions were susceptible or had a low level of resistance to triazophos (RR = 1.00–10.69). Results also showed that most field populations were susceptible to fipronil (RR < 3), but the populations from Ruian and Cangnan, Zhejiang, in 2006 showed moderate levels of resistance to fipronil (RR = 20.99–25.35). All 11 field populations collected in 2002–2006 were susceptible to abamectin (RR < 5). The tolerance levels in the rice stem borer exhibited an increasing trend (or with fluctuation) over a 5-yr period for different insecticides, and they reached a maximal level in 2006 for all four insecticides. Analysis of regional resistance ratios indicated that the history and intensity of insecticide application are the major driving forces for the resistance evolution in *C. suppressalis*. Strategic development of insecticide resistance management also is proposed.

KEY WORDS *Chilo suppressalis*, resistance, triazophos, fipronil, monosultap

The Asiatic rice borer, *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae), an important pest insect of rice, *Oryza sativa* L., in Asia (Konno et al. 1986), occurs in all rice-growing areas in China (Sheng et al. 2003). Control of this insect relies mainly on chemical insecticides. In the past 10 yr, population density and its damaging intensity increased dramatically in China and posed a severe threat to the trait of high and stable yields of the crop (Sheng et al. 2003). Resistance development to insecticides in *C. suppressalis* is one of the major factors for its population increase and infestations (Cao et al. 2003).

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Monosultap, triazophos, fipronil, and abamectin are four major insecticides that have been used to control *C. suppressalis* for the past 10–20 yr. Monosultap, as well as bisultap, a nereistoxin analog found by the Chemical Institution of Guizhou Province in China in 1974, has been the most extensively and intensively used insecticide for controlling *C. suppressalis* since the 1980s (Cao and Shen 2005). Triazophos, an organophosphate insecticide, was first introduced to control monosultap-resistant *C. suppressalis* in early 1990s in Zhejiang Province (Jiang et al. 2001). It gradually replaced monosultap (and bisultap) and became the preferred insecticide for controlling *C. suppressalis* in large rice areas in recent years (Cao et al. 2004). Fipronil, a phenylpyrazole insecticide, was a more recently introduced insecticide for controlling *C. suppressalis* and other rice insects in most rice-growing areas (Cao et al. 2004). Abamectin, a macrocyclic lactone, has been used on rice to control *C. suppressalis* mainly in the form of mixtures with other insecticides since 1998 (Cao et al. 2004).

Due to the high toxicity to high animals and the hazardous risk to the environment, many pesticides, including methamidophos, parathion, methyl-parathion, monocrotophos, and phosphamidon once being major insecticides for rice insect control, are sub-

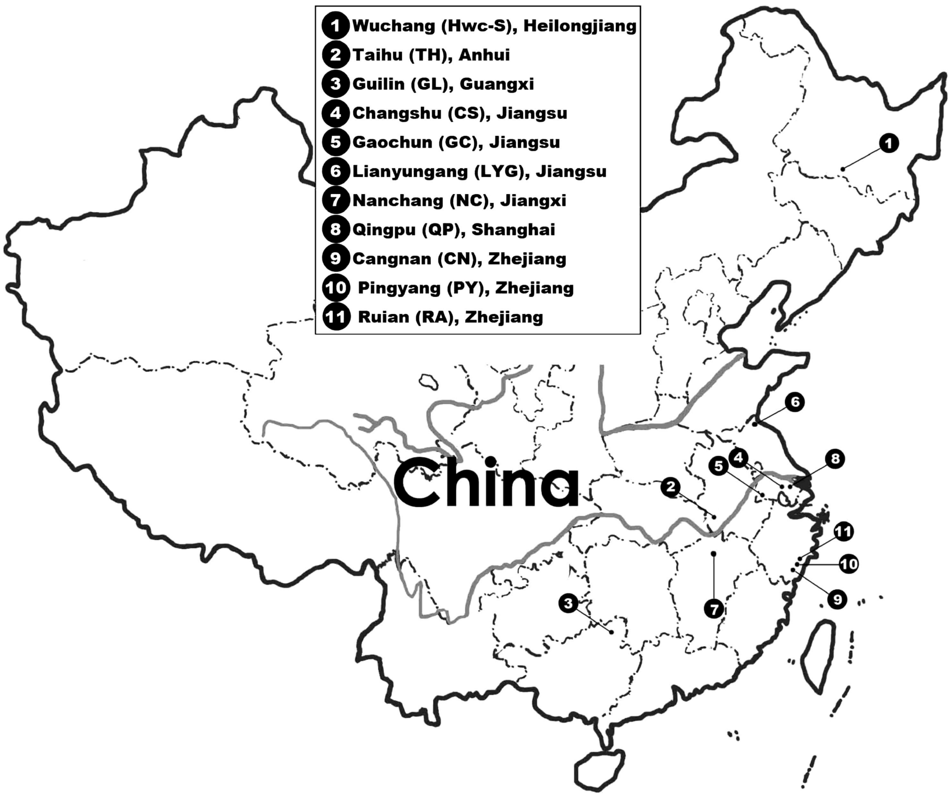

Fig. 1. Map showing collection sites of 11populations of *C. suppressalis* tested in this study.

jected to be phased out entirely in 2007. Monosultap, triazophos, fipronil, and abamectin may potentially become the major chemicals to control the rice stem borer. Previous studies on insecticide resistance in *C. suppressalis* were limited to a few local populations. To provide a foundation for areawide resistance management of rice stem borer, we initiated a study to investigate spatial and temporal changes in susceptibilities to monosultap, triazophos, fipronil, and abamectin to monitor the dynamics of resistance to long-history-use insecticides such as monosultap and triazophos, and to monitor potential resistance development to the newly introduced insecticides fipronil and abamectin.

Materials and Methods

Insects. From 2002 to 2006, 20 samples in total of *C. suppressalis* were collected in rice fields from 11 locations in Anhui, Heilongjiang, Jiangsu, Jiangxi, and Zhejiang provinces, and Guangxi Autonomous Region, and Shanghai Municipality (Fig. 1). Except for two populations, QP03 and GL05, collected from the second and third field generations, respectively, all populations were collected from the first field generation. The Hwc-S population, collected in 2002 from Wuchang, Heilongjiang, where the application level of pesticides for controlling this pest was extremely low, was used as the susceptible population. All field populations were collected during egg stage. More than 100 egg masses were collected in each population, and average size of the egg mass was ≈100 eggs. All insects were reared in the laboratory by using a rice seedling rearing method (Shang et al. 1979), fourth instars were directly used for bioassays. Rearing conditions were maintained at 28 ± 1°C and a photoperiod of 16:8 (L:D) h.

Insecticides. The following technical grade insecticides were used in bioassays: monosultap (Jiangsu Liyang Chemical Factory, Liyang, China, 90% active ingredient [A.I.]), triazophos (Zhejiang Yongnong Chem. Ind. Co., Ltd., Wenzhou, China, 80.5% [A.I.]), fipronil (Aventis and Bayer CropScience Hangzhou Co., Ltd., Hangzhou, China, 87% [A.I.]), and abamectin (North China Pharmaceutical Co., Ltd., Shijiazhuang, China, 97% [A.I.]).

Bioassays. The topical application method (FAO 1980) was used to conduct bioassay on each population of *C. suppressalis.* Middle fourth instars with body weight ranging 6–9 mg per larva were used as a standard larval stage in the bioassays (Cao et al. 2001). Larvae were placed into petri dishes (5 cm) containing a piece (1 by 1 by 0.3 cm) of artificial diet. The components of the artificial diet reported by Tan (1987) were revised from the recipe reported by FAO (1980). Insecticides were diluted into a series of concentrations with acetone (five to seven insecticide doses and a control were normally included in each

Table 1. Dose responses and resistance ratios of field populations of *C. suppressalis* to abamectin and fipronil

Pop	Yr	Abamectin					Fipronil				
		n^a	Slope ± SE	LD_{50} ± SE (ng/larva)	χ^2	RR ± SE[b]	n^a	Slope ± SE	LD_{50} ± SE (ng/larva)	χ^2	RR ± SE[b]
Hwc-S	2002	210	3.07 ± 0.46	0.16 ± 0.02	3.10	1.00 ± 0.11ij	210	4.97 ± 0.68	0.91 ± 0.19	0.88	1.00 ± 0.21jkl
TH, Anhui	2002	205	2.11 ± 0.33	0.17 ± 0.01	4.69	1.04 ± 0.08hij	170	4.11 ± 0.61	1.30 ± 0.10	1.40	1.43 ± 0.11ijk
	2003	180	3.39 ± 0.48	0.14 ± 0.01	5.94	0.86 ± 0.06j	195	2.04 ± 0.33	1.53 ± 0.09	0.21	1.69 ± 0.10hij
GL, Guangxi	2005	210	2.96 ± 0.37	0.47 ± 0.02	1.09	2.94 ± 0.10bc	210	4.10 ± 0.59	0.83 ± 0.05	1.76	0.91 ± 0.05jkl
CS, Jiangsu	2002	175	2.76 ± 0.46	0.25 ± 0.01	13.02	1.54 ± 0.06g	210	2.83 ± 0.45	1.83 ± 0.13	10.04	2.02 ± 0.15ghi
	2004	180	1.92 ± 0.63	0.22 ± 0.02	3.45	1.40 ± 0.09ghi	210	1.86 ± 0.25	0.39 ± 0.03	3.60	0.42 ± 0.03l
GC, Jiangsu	2006	150	2.56 ± 0.48	0.54 ± 0.02	1.89	3.37 ± 0.09ab	210	2.74 ± 0.48	2.39 ± 0.21	9.42	2.63 ± 0.23gh
LYG, Jiangsu	2002	140	3.86 ± 0.61	0.12 ± 0.00	3.49	0.75 ± 0.00j	179	3.96 ± 0.60	0.92 ± 0.04	1.37	1.01 ± 0.05jkl
	2005	174	3.74 ± 0.65	0.24 ± 0.04	4.08	1.52 ± 0.25gh	210	2.55 ± 0.35	0.52 ± 0.13	5.97	0.57 ± 0.14kl
	2006	150	2.65 ± 0.45	0.34 ± 0.04	2.98	2.11 ± 0.23ef	124	2.18 ± 0.47	1.53 ± 0.12	5.23	1.69 ± 0.13ij
NC, Jiangxi	2005	168	2.65 ± 0.38	0.04 ± 0.01	4.22	0.23 ± 0.03k	144	3.58 ± 0.53	0.63 ± 0.08	4.92	0.70 ± 0.09kl
QP, Shanghai	2003	235	1.08 ± 0.54	0.41 ± 0.07	2.74	2.54 ± 0.42cde	355	2.44 ± 0.38	2.53 ± 0.09	8.91	2.29 ± 0.10g
	2004	210	1.89 ± 0.51	0.35 ± 0.06	1.89	2.19 ± 0.34e	181	4.17 ± 0.70	0.83 ± 0.10	1.34	0.91 ± 0.11jkl
CN, Zhejiang	2003	210	3.14 ± 0.46	0.27 ± 0.02	1.64	1.69 ± 0.10fg	210	8.75 ± 0.77	15.53 ± 0.23	6.12	17.07 ± 0.26c
	2006	211	3.18 ± 0.45	0.61 ± 0.01	4.64	3.79 ± 0.04a	150	3.95 ± 0.60	23.07 ± 0.91	1.357	25.35 ± 0.99a
PY, Zhejiang	2003	175	2.57 ± 0.42	0.37 ± 0.03	1.01	2.29 ± 0.21de	200	10.33 ± 1.45	13.00 ± 0.35	2.69	14.29 ± 0.38d
RA, Zhejiang	2002	210	3.71 ± 0.53	0.34 ± 0.01	0.63	2.15 ± 0.06ef	210	3.04 ± 0.42	8.60 ± 0.10	1.28	9.45 ± 0.11f
	2003	210	2.85 ± 0.31	0.27 ± 0.01	1.24	1.69 ± 0.06fg	245	3.11 ± 0.39	9.43 ± 0.50	11.53	10.37 ± 0.55ef
	2005	186	2.00 ± 0.24	0.33 ± 0.02	3.91	2.06 ± 0.13ef	150	3.58 ± 0.55	9.93 ± 0.41	4.28	10.92 ± 0.45e
	2006	240	2.41 ± 0.34	0.44 ± 0.03	1.85	2.77 ± 0.19cd	180	3.07 ± 0.51	19.10 ± 0.45	2.43	20.99 ± 0.50b

[a] Number of insects tested.
[b] Means followed by same letters are not significantly different at $P = 0.05$ within column.

bioassay), except monosultap with a mixture of acetone and water at a ratio of 1:1 because of its low solubility in acetone. A droplet of 0.04 μl of insecticide solution was applied topically on the dorsal part of larval middle abdomen with a capillary microapplicator (FAO 1980). Three replicates were used and in each replication, 10 larvae were treated for each insecticide concentration. Control insects were treated with acetone alone or with a mixture of acetone and water as control for the treatments of monosultap. The rearing conditions for treated larvae were controlled at 28 ± 1°C and a photoperiod of 16:8 (L:D) h. Mortality was recorded 48 h after treatment for triazophos, 72 h for fipronil, and 96 h for monosultap and abamectin. The time length for post treatment mortality counting was determined based on effectiveness of each insecticide. Larvae were counted as dead if no response was observed after being probed with a pin.

Statistical Analysis. The PoloPlus software (LeOra Software 2002) was used for probit analysis of dose–response data. The resistance ratio (RR) was calculated by dividing the LD_{50} of a field population by the corresponding LD_{50} of the susceptible strain (Hwc-S). Resistance levels were classified based on Shen's standard (Shen and Wu 1995) as susceptible, RR < 3; minor resistance, RR = 3–5; low resistance level, RR = 5–10; medium resistance level, RR = 10–40; high resistance level: RR = 40–160; and extremely high resistance level: RR > 160. Data were further statistically analyzed with SAS program (SAS Institute 1990). PROC MIXED and PROC GLM procedures were used for variance analyses. Mean separation was conducted using SAS PROC MEANS/least significant difference (LSD) or LSmeans separation programs at $P < 0.05$.

Results

Variations of Dose Response and Resistance Ratios among Four Insecticides. Susceptibilities to abamectin, fipronil, monosultap, and triazophos in *C. suppressalis* collected from a total of 11 field populations in seven provinces (municipality, or autonomous region) were evaluated from 2002 to 2006. The dose–response data and the RRs of field populations of *C. suppressalis* to four insecticides were listed in Tables 1 and 2. The baseline toxicities of abamectin, fipronil, triazophos, and monosultap to the susceptible strain (Hwc-S) were 0.16 ± 0.02, 0.91 ± 0.19, 6.23 ± 0.28, and 286.57 ± 16.46 ng per larva, respectively. Monosultap had the lowest efficacy against *C. suppressalis*, which was significantly different from those of the other three insecticides ($F = 297.98$, df = 3, $P < 0.0001$).

Pooled RR data from 11 populations indicated significantly different resistance levels to four insecticides ($F = 15.89$, df = 3, $P < 0.0001$). A highest level of resistance was developed to triazophos (mean RR = 149.95), a medium level of resistance to monosultap (RR = 49.09), a low level of resistance to fipronil (RR = 6.31), and all the populations were still very susceptible to abamectin (RR = 1.90).

Variations of Resistance Ratios among Populations for Each Insecticide. *Abamectin.* Susceptibilities to abamectin in field populations from seven provinces (municipality, or autonomous region) were evaluated from 2002 to 2006 (Table 1; Fig. 2A). Although the resistance ratios were significantly different among 20 samples ($F = 28.24$, df = 19, $P < 0.0001$), all populations were still susceptible to abamectin (RR < 5).

Fipronil. Resistance ratios to fipronil in *C. suppressalis* were significantly different among 20 different field samples ($F = 545.73$, df = 19, $P < 0.0001$) (Table

Table 2. Dose responses and resistance ratios of field populations of *C. suppressalis* to monosultap and triazophos

Pop	Yr	Monosultap					Triazophos				
		n^a	Slope ± SE	LD_{50} ± SE (ng/larva)	χ^2	RR ± SE[b]	n^a	Slope ± SE	LD_{50} ± SE (ng/larva)	χ^2	RR ± SE[b]
Hwc-S	2002	240	2.15 ± 0.27	286.57 ± 16.46	0.31	1.00 ± 0.06g	200	3.13 ± 0.43	6.23 ± 0.29	1.57	1.00 ± 0.04e
TH, Anhui	2002	160	2.45 ± 0.34	3,285.93 ± 487.75	0.90	11.47 ± 1.70g	395	2.83 ± 0.27	7.60 ± 0.23	7.98	1.22 ± 0.03e
	2003	185	2.40 ± 0.33	3,375.13 ± 286.02	3.08	11.78 ± 1.00g	150	4.56 ± 0.65	8.47 ± 0.63	0.87	1.36 ± 0.10de
GL, Guangxi	2005	171	2.63 ± 0.49	3,307.50 ± 392.46	2.66	11.54 ± 1.37g	180	1.94 ± 0.30	327.53 ± 66.39	4.28	52.57 ± 10.66e
CS, Jiangsu	2002	243	2.16 ± 0.29	4,207.73 ± 207.73	2.97	14.69 ± 0.73fg	286	2.16 ± 0.31	66.63 ± 7.77	3.84	10.69 ± 1.25e
	2004	144	3.04 ± 0.51	2,593.13 ± 113.21	0.06	9.05 ± 0.39g	150	3.63 ± 0.53	44.88 ± 2.01	4.30	7.20 ± 0.32e
GC, Jiangsu	2006	150	1.01 ± 0.49	62,910.00 ± 5,477.75	0.83	219.53 ± 19.12a	210	1.31 ± 0.23	361.83 ± 70.36	3.31	58.08 ± 11.29de
LYG, Jiangsu	2002	207	1.76 ± 0.28	1,359.43 ± 94.87	4.98	4.75 ± 0.33g	146	2.20 ± 0.42	4.83 ± 0.36	0.28	0.78 ± 0.06e
	2005	180	1.40 ± 0.29	1,865.97 ± 804.87	2.73	6.15 ± 2.81g	180	3.54 ± 0.39	6.83 ± 1.42	6.81	1.10 ± 0.23e
	2006	155	1.81 ± 0.35	2,563.47 ± 278.64	1.16	8.94 ± 0.97g	155	2.23 ± 0.76	9.07 ± 0.92	4.06	1.45 ± 0.15e
NC, Jiangxi	2005	120	2.59 ± 0.58	3,030.13 ± 647.97	0.80	10.57 ± 2.26g	120	2.20 ± 0.48	126.67 ± 11.94	0.83	20.33 ± 1.92e
QP, Shanghai	2003	308	2.02 ± 0.23	3,358.27 ± 186.48	5.75	11.72 ± 0.65g	211	1.76 ± 0.27	37.57 ± 1.51	5.38	6.03 ± 0.24e
	2004	180	1.75 ± 0.41	8,849.70 ± 127.50	0.65	30.88 ± 0.45f	180	2.53 ± 0.31	25.77 ± 3.62	1.12	4.14 ± 0.58e
CN, Zhejiang	2003	165	2.46 ± 0.39	21,545.70 ± 1,110.18	3.53	75.19 ± 3.87d	250	1.64 ± 0.21	3675.30 ± 179.39	8.53	589.93 ± 28.79b
	2006	193	3.01 ± 0.46	31,886.80 ± 2,706.57	6.70	111.27 ± 9.44c	328	1.68 ± 0.21	5576.27 ± 336.80	0.86	895.07 ± 54.06a
PY, Zhejiang	2003	200	2.03 ± 0.23	16,322.43 ± 1,424.39	0.26	56.96 ± 4.97e	210	2.43 ± 0.31	2843.73 ± 68.50	4.68	456.46 ± 11.00c
RA, Zhejiang	2002	175	1.77 ± 0.38	32,995.00 ± 1,912.37	1.28	115.14 ± 6.67c	210	1.77 ± 0.27	1006.63 ± 17.43	1.48	161.58 ± 2.80d
	2003	175	2.70 ± 0.46	21,457.17 ± 82.87	0.33	74.88 ± 0.29d	210	1.95 ± 0.31	538.77 ± 49.72	1.24	86.48 ± 7.98de
	2005	120	2.71 ± 0.45	15,661.03 ± 2,544.48	1.04	54.65 ± 8.88e	192	1.83 ± 0.24	472.63 ± 86.19	2.61	75.86 ± 13.83de
	2006	177	1.70 ± 0.31	40,466.80 ± 1,018.10	2.97	141.21 ± 3.55b	240	1.29 ± 0.19	3536.50 ± 1016.12	2.61	567.66 ± 163.10bc

[a] Number of insects tested.
[b] Means followed by same letters are not significantly different at $P = 0.05$ within column.

1), among 11 different populations sampled from 2002 to 2006 ($F = 37.82$, df = 10, $P < 0.0001$) (Fig. 2A), and among seven different provinces ($F = 35.70$, df = 6, $P < 0.0001$). Three populations (CN, PY, and RA) from southeastern Zhejiang Province had developed a medium level of resistance to fipronil (RR = 21.21, 14.29 and 12.93, respectively), whereas other populations were still susceptible to fipronil (RR < 3) (Table 1; Fig. 2A).

Monosultap. Most field populations of *C. suppressalis* were resistant to monosultap (RR = 6.73–219.53) (Table 2; Fig. 2B). High levels of resistance were detected in three populations (CN, PY, and RA) from southeastern Zhejiang Province (RR = 93.25, 56.96, and 96.47, respectively) and in GC population from Jiangsu Province (RR = 219.53). Resistance ratios to monosultap were significantly different among 20 different field samples ($F = 105.22$, df = 19, $P < 0.0001$), among 11 different populations collected in 5 yr ($F = 43.48$, df = 10, $P < 0.0001$), and among seven different provinces ($F = 4.19$, df = 6, $P < 0.005$ [0.0016]).

Triazophos. Some *C. suppressalis* populations in TH, Anhui, LYG and CS, Jiangsu, and QP, Shanghai were susceptible or resistant with low levels to triazophos (RR < 10) (Table 2; Fig. 2B), whereas some other populations from GL, Guangxi, GC, Jiangsu, NC, Jiangxi, and CX, Zhejiang were resistant to triazophos with medium or high levels (RR = 20.33–66.89). In southeastern Zhejiang Province, resistance of *C. suppressalis* to triazophos had reached an extremely high level. The CN, PY, and RA populations had RRs up to 742.50-, 456.46-, and 222.89-fold, respectively. Resistance ratios to triazophos were significantly different among 20 different field samples ($F = 43.35$, df = 19, $P < 0.0001$), among 11 different populations collected in 5 yr ($F = 19.26$, df = 10, $P < 0.0001$), and among seven different provinces ($F = 9.72$, df = 6, $P < 0.0001$).

Variations of Resistance Ratios among Different Years. Resistances to four insecticides in LYG and RA populations were evaluated in five consecutive years (2002–2006) (Fig. 3). LYG population had only developed a low level of resistance to monosultap (Fig. 3C), and it showed no significant year-to-year difference ($F = 1.34$, df = 3, $P > 0.05$ [0.3278]). Except for being susceptible to abamectin (Fig. 3A), the RA population had developed different resistance levels to fipronil, monosultap, and triazophos. This population exhibited greater year-to-year variation in resistance ratios to fipronil, monosultap, and triazophos (Fig. 3B–D). The resistance level to fipronil increased from 2.27-fold in 2001–20.99-fold in 2006 ($F = 288.23$, df = 4, $P < 0.0001$). The resistance level to monosultap ranged from 54.65-fold in 2005 to 141.21-fold in 2006 ($F = 44.73$, df = 3, $P < 0.0001$). Similarly, the resistance ratios to triazophos fluctuated from 38.67-fold in 2004 to 567.66-fold in 2006 ($F = 8.84$, df = 4, $P < 0.005$).

Discussion

In this study, variable susceptibilities to four selected insecticides were detected in different populations of *C. suppressalis.* The insect tended to increase its tolerance during the past 5-yr period. The rates of resistance increase varied substantially for different insecticides and for different regions. Our bioassay results indicated that in *C. suppressalis,* the changes of the susceptibilities to monosultap, triazophos, fipronil, and abamectin are apparently correlated to the selection pressure applied by variable insecticides, application history, and selection intensity in different regions. The results from this study seem to agree with the evolutionary theory of insecticide resistance development (Roush and Daly 1990) driven mainly by the selection of insecticides (Palumbi 2001).

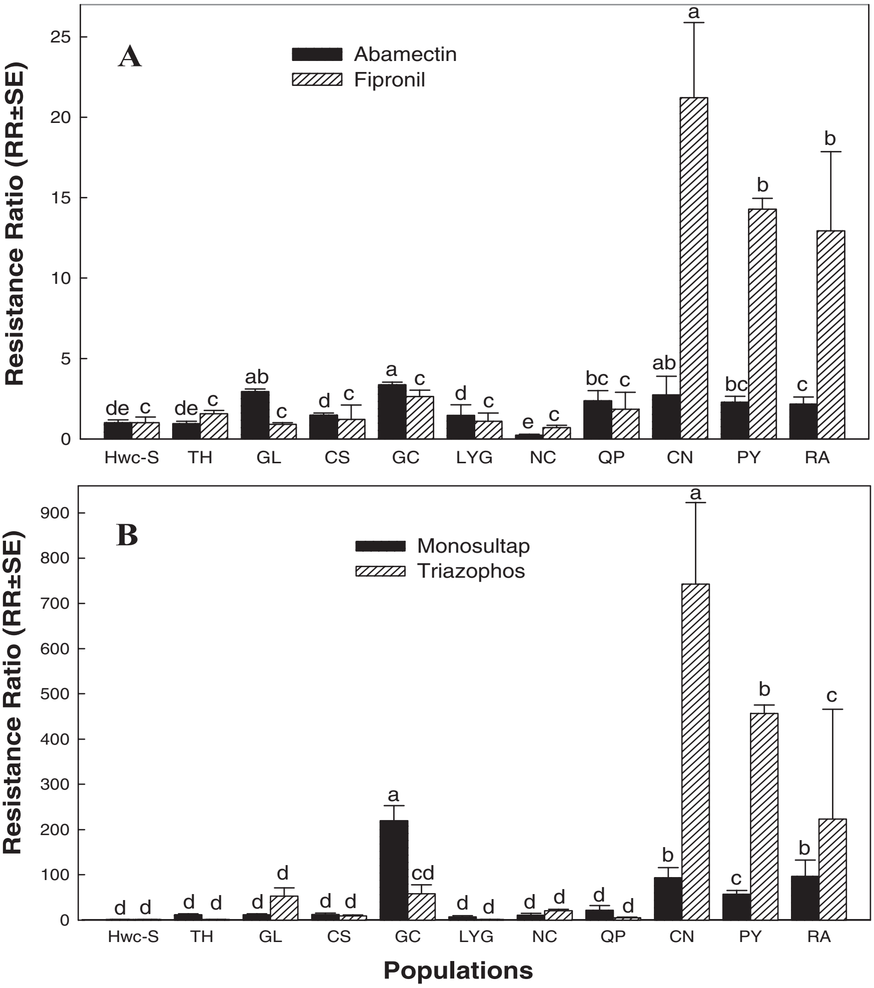

Fig. 2. Variations of resistance ratios to abamectin and fipronil (A) and monosultap and triazophos (B) in 11 *C. suppressalis* populations (The bars topped with the same letters are not significantly different at $P < 0.05$ within insecticide: Hwc-S, the susceptible strain; TH, Taihu, Anhui; GL, Guilin, Guangxi; CS, Changshu, Jiangsu; GC, Gaochun, Jiangsu; LYG, Lianyungang, Jiangsu; NC, Nanchang, Jiangxi; QP, Qingpu, Shanghai; CN, Cangnan, Zhejiang; PY, Pingyang, Zhejiang; RA, Ruian, Zhejiang).

Currently, control of *C. suppressalis* relies almost exclusively on insecticides. Pesticide resistance development in this insect has become a serious issue. In the past, methamidophos, a highly toxic organophosphate insecticide, and bisultap (monosultap) were the major insecticides used for *C. suppressalis* control. Afterward, triazophos came to be extensively used along with some new high-efficacy insecticides, such as fipronil or abamectin (Qu et al. 2003). Phasing out highly toxic organophosphate insecticides has created a shortage of chemical selection and leaves farmers with no choice other than relying exclusively on a few insecticides, such as monosultap and triazophos. As a consequence of high selection pressure on target insect, a lack of diversity and improper use of insecticides might have prompted resistance development in some rice production areas.

Monosultap has been extensively and intensively applied for controlling *C. suppressalis* for ≈20 yr (Jiang et al. 2001). Since 1998, high levels of resistance to monosultap in some field populations of *C. suppressalis,* especially from Zhejiang Province, have been observed (Su et al. 1996, Cao et al. 2001, Lu et al. 2003, Wang et al. 2004, Xiong et al. 2004, Liu et al. 2005, Zhou 2005). The results from this study also indicated that the resistance to monosultap in *C. suppressalis* is becoming widespread across the rice production areas in China. Triazophos has an application history of >10 yr in some rice-growing areas in China. Consequently, high levels of resistance to triazophos have been detected since 1999 in some populations from southeast Zhejiang Province. Subsequently, some populations in southern Jiangsu, Jiangxi, and Guangxi provinces have developed high levels of resistance to triazophos (Cao et al. 2001, Jiang et al. 2001, Qu et al. 2003, Huang et al. 2005). The resistance development to monosultap and triazophos was potentially prompted by intensity of insecticide applications. In the southeastern Zhe-

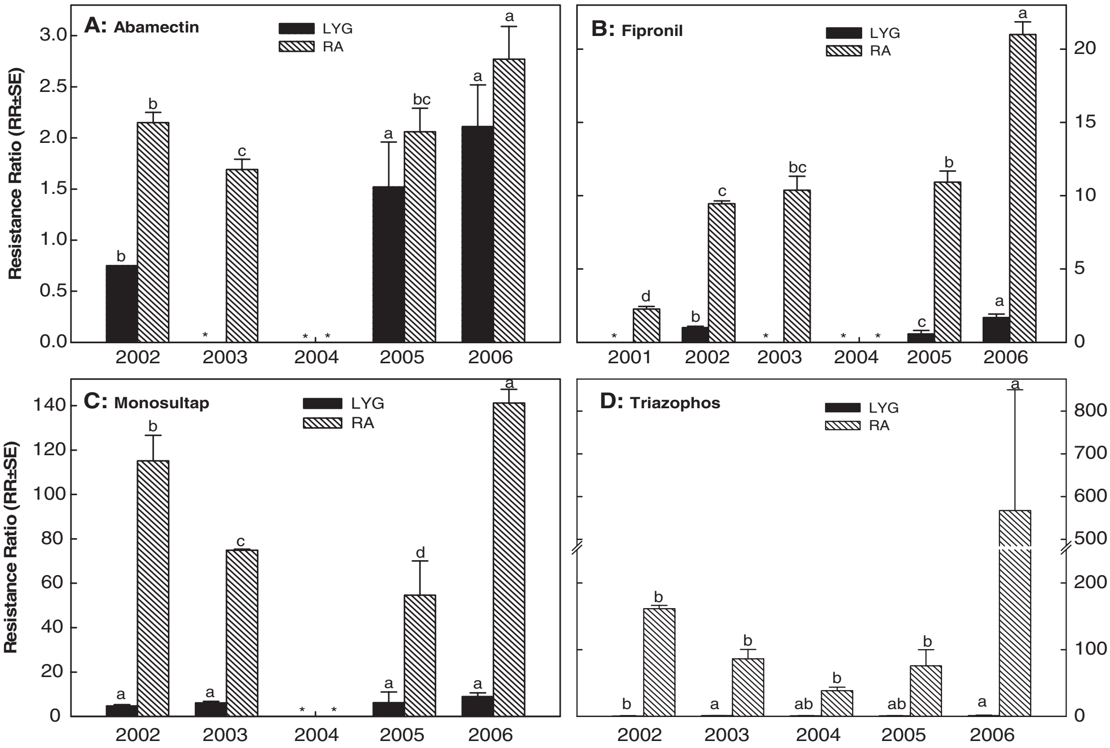

Fig. 3. Changes of resistance ratios to abamectin (A), fipronil (B), monosultap (C), and triazophos (D) during the past 5 yr (2002–2006) in LYG and RA populations (asterisk [*], data are not available. The bars topped with the same letters are not significantly different at $P < 0.05$ within insecticide (LYG, Lianyungang, Jiangsu; RA, Ruian, Zhejiang).

jiang Province, *C. suppressalis* has four generations a year, and insecticide use was the highest in the nation. As a consequence, the resistance to triazophos and monosultap was also at the highest level. However, some other populations of *C. suppressalis* in Anhui, Jiangxi, and Hubei provinces were relatively susceptible or had a low level of triazophos resistance (Wang et al. 2004, Xiong et al. 2004, Liu et al. 2005, Zhou 2005) because of relatively low-level insecticide applications in those areas.

Fipronil has been applied extensively in southeastern rice areas of Zhejiang Province since ≈1997 (Cao et al. 2004, Jiang et al. 2005). Consequently, in 2002, resistance to fipronil was first found in a field population from RA, Zhejiang (Cao et al. 2004), and since then, the resistance level has increased to nearly 20-fold in 2006, as detected in this study. A regional survey of rice-growing areas in southeastern China (Jiang et al. 2005) indicated that the Asiatic rice borer was still susceptible to fipronil in 2001. Subsequent surveys of one local population (Cangnan, Zhejiang) from 2002 to 2004 (Jiang et al. 2005) showed that the population had different susceptibility (RR = 8.2–21.2), which was similar to the level we detected in this study. Our comprehensive surveys from 2002 to 2006 indicated that the Asiatic rice borer in Zhejiang province, including the Cangnan population, was more resistant to fipronil than any populations from other provinces. The resistance levels tended to increase over the 4-yr period, especially the RA population. Year-to-year comparison of the sensitivities in this population provided evidence of increasing fipronil resistance levels in *C. suppressalis.* Although most other field populations of *C. suppressalis* remain susceptible to fipronil, resistance to fipronil might become more prominent and serious if it is used continually and extensively without proper resistance management strategies. Abamectin has been used mainly in the form of mixtures with other insecticides to control *C. suppressalis* since 1998 in some locations (Cao et al. 2004). As a result of relatively weak selection pressure on field populations, no high level resistance to abamectin has been detected in *C. suppressalis.* However, the potential for resistance evolution cannot be ignored and it is necessary to continue monitoring the susceptibility in field population of *C. suppressalis.*

The populations also showed a general increase in resistance ratios during the 5-yr period (2002–2006) to all insecticides. Year-to-year comparisons did not reveal a consistently increasing rate every year. Instead, year-to-year fluctuations of resistance ratios were common for most insecticides. For example, the RA population exhibited fluctuating variation in resistance ratios to monosultap and triazophos (Fig. 3C and D). The tolerance levels in this population, however, reached a maximal level in 2006 for all four insecticides. Many factors might contribute to fluctuating variation of the resistance ratio. One of the possible factors was the frequency and method of insecticide deployment under field conditions. Field surveys showed that the fluctuations of the resistance over 5 yr in RA population were possibly correlated with insecticide applications in local fields. Because a high level of resistance to monosultap and triazophos was detected in 2002, the application levels of these two insecticides were reduced due to the practice of rotation or mixtures (communication with local plant protection stations) with other insecticides. Thus, the resistance levels to these two insecticides showed a decrease until 2004, when these two insecticides again became the dominant insecticides used for *C. suppressalis* control. The shortage of chemical selection was created by the relevant authority for phasing out highly toxic organophosphate insecticides and left farmers with little choice of chemicals for this insect. Consequently, the resistance levels to monosultap and triazophos have increased since 2004. This phenomenon also indicated that the evolution of insecticide resistance in *C. suppressalis* is driven mainly by the localized selection pressures.

Based on the current study on resistance development, we also observed that the resistance ratios in *C. suppressalis* varied significantly from region to region and varied from insecticide to insecticide. Therefore, chemical control plans and resistance management strategies must be developed to fit each rice-growing region. First, tracking resistance development based on insecticide application history and intensity in a particular location is important for directing effective and sustainable use of insecticides. Relevant recommendations for *C. suppressalis* resistance management for different rice-growing regions must be developed accordingly based on chemical control history to fit each particular rice-growing region.

One important measure to manage insecticide resistance and to delay resistance development is to modify pesticide use by using sequences, alternations, rotations, or mixtures of insecticides that have no cross-resistance between each other (Tabashnik 1989). Such tactics are recommended by the Insecticide Resistance Action Committee (IRAC) (www.irac-online.org). The use of mixtures is only recommended where there is no resistance to either component of the mixture; otherwise, alternation is a better strategy. Monosultap, triazophos, fipronil, and abamectin, the four major insecticides for *C. suppressalis* control in recent years, are all different from each other in mode of action based on the IRAC mode of action classification scheme. In addition, metabolic cross-resistance between these insecticides has not been identified. Accordingly, as expected, cross-resistance between them has not been found, as indirectly indicated from our bioassay data in this study and partly tested in a previous study (Cao 2004), which showed that the high triazophos-resistant strain (RR = 1,636.1-fold) obtained from laboratory selection had no cross-resistance to monosultap and fipronil. The toxicity data in that work indicated that field populations with high levels of resistance to monosultap and triazophos were very sensitive to fipronil or abamectin, such as the field populations from GC, Jiangsu and RA, Zhejiang collected in 2006.

Although this study provided a systemic surveillance of resistance levels to four widely used insecticides on *C. suppressalis* and a potential connection between resistance severity and insecticide application intensity, several issues need to be studied before a sound resistance management program can be developed and implemented. First, it is important to study the nature of the resistance to determine whether the resistance is dominant or recessive and which mechanisms are involved in the resistance. This study could lead to selection and application of specific inhibitors against target enzymes which are responsible for the resistance. To understand resistance mechanisms also might lead to development of rapid and useful diagnostic assays (insecticidal, biochemical, and molecular) for monitoring resistance evolution in the future. Second, it is necessary to investigate whether host plant or rice variety has an influence on *C. suppressalis* population density and resistance development. Understanding host plant impacts is important because of the potential for using resistant rice varieties and transgenic rice for management of *C. suppressalis* populations in the future. Third, analysis of geographic and climatic differences among rice production regions needs to be considered in the future to determine key factors causing fluctuation of the resistance in *C. suppressalis*, which was observed in this study. Findings of the current study also may be applied in the development of fitness assays for understanding costs of resistance in resistant populations. And finally, to determine how the resistance changes from generation to generation over a season is also important for development of a successful resistance management program.

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